TITLE

4,4-DISUBSTITUTED-3,4-DIHYDRO-2(1H)-QUINAZOLINIONES USEFUL AS HIV REVERSE TRANSCRIPTASE INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the priority benefit of U.S. Provisional Application No. 60/400,409, filed August 1, 2002, which is expressly incorporated fully herein by reference.

FIELD OF THE INVENTION

This invention relates generally to quinazolinones compounds and also quinazolinones compounds which are useful as inhibitors of HIV reverse transcriptase, pharmaceutical compositions and diagnostic kits comprising the same, methods of using the same for treating viral infection or as assay standards or reagents, and intermediates and processes for making such quinazolinones compounds.

BACKGROUND OF THE INVENTION

Two distinct retroviruses, human immunodeficiency virus (HIV) type-1 (HIV-1) or type-2 (HIV-2), have been etiologically linked to the immunosuppressive disease, acquired immunodeficiency syndrome (AIDS). HIV seropositive individuals are initially asymptomatic but typically develop AIDS related complex (ARC) followed by AIDS. Affected individuals exhibit severe immunosuppression which predisposes them to debilitating and ultimately fatal opportunistic infections.

The disease AIDS is the consequence of HIV-1 or HIV-2 virus following its complex viral life cycle. The virion life cycle involves the virion attaching itself to the host human T-4 lymphocyte immune cell through the binding of a glycoprotein on the surface of the virion's protective coat with the CD4 glycoprotein on the lymphocyte cell. Once attached, the virion sheds its

glycoprotein coat, penetrates into the membrane of the host cell, and uncoats its RNA. The virion enzyme, reverse transcriptase, directs the process of transcribing the RNA into single-stranded DNA. The viral RNA is degraded and a second DNA strand is created. The now double-stranded DNA is integrated into the human cell's genes and those genes are used for virus reproduction.

RNA polymerase transcribes the integrated viral DNA into viral mRNA. The viral RNA is translated into the precursor *gag-pol* fusion polyprotein. The polyprotein is then cleaved by the HIV protease enzyme to yield the mature viral proteins. Thus, HIV protease is responsible for regulating a cascade of cleavage events that lead to the virus particle's maturing into a virus that is capable of full infectivity.

The typical human immune system response, killing the invading virion, is taxed because the virus infects and kills the immune system's T cells. In addition, viral reverse transcriptase, the enzyme used in making a new virion particle, is not very specific, and causes transcription mistakes that result in continually changed glycoproteins on the surface of the viral protective coat. This lack of specificity decreases the immune system's effectiveness because antibodies specifically produced against one glycoprotein may be useless against another, hence reducing the number of antibodies available to fight the virus. The virus continues to reproduce while the immune response system continues to weaken. In most cases, without therapeutic intervention, HIV causes the host's immune system to be debilitated, allowing opportunistic infections to set in. Without the administration of antiviral agents, immunomodulators, or both, death may result.

There are at least three critical points in the HIV life cycle which have been identified as possible targets for antiviral drugs: (1) the initial attachment of the

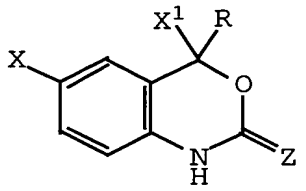
virion to the T-4 lymphocyte or macrophage site, (2) the transcription of viral RNA to viral DNA (reverse transcriptase, RT), and (3) the processing of gag-pol protein by HIV protease.

Inhibition of the virus at the second critical point, the viral RNA to viral DNA transcription process, has provided a number of the current therapies used in treating AIDS. This transcription must occur for the virion to reproduce because the virion's genes are encoded in RNA and the host cell transcribes only DNA. By introducing drugs that block the reverse transcriptase from completing the formation of viral DNA, HIV-1 replication can be stopped.

A number of compounds that interfere with viral replication have been developed to treat AIDS. For example, nucleoside analogs, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxythymidinene (d4T), 2',3'-dideoxyinosine (ddI), and 2',3'-dideoxy-3'-thia-cytidine (3TC) have been shown to be relatively effective in certain cases in halting HIV replication at the reverse transcriptase (RT) stage.

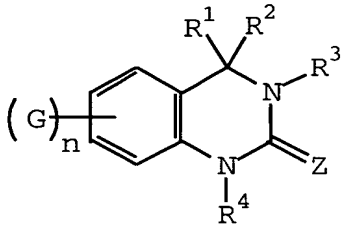
An active area of research is in the discovery of non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs). As an example, it has been found that certain benzoxazinones and quinazolinones are active in the inhibition of HIV reverse transcriptase, the prevention or treatment of infection by HIV and the treatment of AIDS.

U.S. 5,874,430 describes benzoxazinone non-nucleoside reverse transcriptase inhibitors for the treatment of HIV. U.S. 5,519,021 describe non-nucleoside reverse transcriptase inhibitors which are benzoxazinones of the formula:



wherein X is a halogen, Z may be O.

EP 0,530,994 and WO 93/04047 describe HIV reverse transcriptase inhibitors which are quinazolinones of the formula (A):



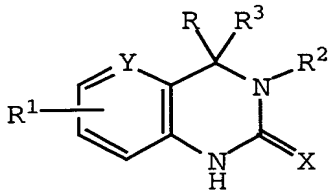
(A)

wherein G is a variety of groups, R^3 and R^4 may be H, Z may be O, R^2 may be unsubstituted alkyl, unsubstituted alkenyl, unsubstituted alkynyl, unsubstituted cycloalkyl, unsubstituted heterocycle, and optionally substituted aryl, and R^1 may be a variety of groups including substituted alkyl.

WO 95/12583 also describes HIV reverse transcriptase inhibitors of formula A. In this publication, G is a variety of groups, R^3 and R^4 may be H, Z may be O, R^2 is substituted alkenyl or substituted alkynyl, and R^1 is cycloalkyl, alkynyl, alkenyl, or cyano. WO 95/13273 illustrates the asymmetric synthesis of one of the compounds of WO 95/12583, (S)-(-)-6-chloro-4-cyclopropyl-3,4-dihydro-4((2-pyridy)ethynyl)-2(1H)-quinazolinone.

Synthetic procedures for making quinazolinones like those described above are detailed in the following references: Houpis et al., *Tetr. Lett.* **1994**, *35(37)*, 6811-6814; Tucker et al., *J. Med. Chem.* **1994**, *37*, 2437-2444; and, Huffman et al., *J. Org. Chem.* **1995**, *60*, 1590-1594.

DE 4,320,347 illustrates quinazolinones of the formula:



wherein R is a phenyl, carbocyclic ring, or a heterocyclic ring. Compounds of this sort are not considered to be part of the present invention.

Even with the current success of reverse transcriptase inhibitors, some patients fail therapy with the development of viral strains that are resistant to a given antiviral regimen. Thus, there is an important ongoing need to develop additional inhibitors to further combat these resistant strains of HIV infection.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides novel reverse transcriptase inhibitors.

The present invention provides novel 4,4-disubstituted-3,4-dihydro-2(1H)-quinazolinone compounds.

The present invention provides novel methods for treating HIV infection which comprises administering to a host in need of such treatment a therapeutically effective amount of the compound of the present invention, including a pharmaceutically acceptable salt form thereof.

The present invention provides novel methods for treating HIV infection which comprises administering to a host in need thereof a therapeutically effective combination of (a) at least one of the compounds of the present invention and (b) one or more compounds selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors.

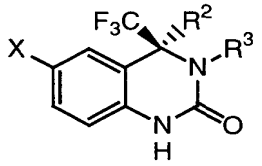
The present invention provides pharmaceutical compositions with reverse transcriptase inhibiting

activity comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of at least one of the compounds of the present invention or a pharmaceutically acceptable salt form thereof.

The present invention provides novel 4,4-disubstituted-3,4-dihydro-2(1H)-quinazolinone compounds for use in therapy.

The present invention provides the use of novel 4,4-disubstituted-3,4-dihydro-2(1H)-quinazolinone compounds for the manufacture of a medicament for the treatment of HIV infection.

These and other aspects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that compounds of formula (I):

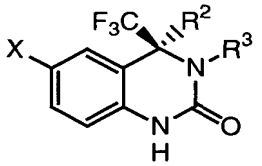


(I)

including any stereoisomeric form, mixtures of stereoisomeric forms, complexes, prodrug forms or pharmaceutically acceptable salt forms thereof, are effective reverse transcriptase inhibitors.

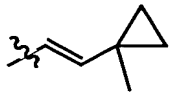
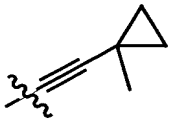
DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[1] Thus, in a first embodiment, the present invention provides a novel compound of formula (I):



(I)

or a stereoisomeric form, mixtures of stereoisomeric forms, complexes, prodrug forms or pharmaceutically acceptable salt form thereof, wherein

R^2 is selected from  and  ;

R^3 is selected from C_{1-6} alkyl and cyclopropyl; and

X is selected from F, Cl, Br, and I.

[2] In another embodiment, the present invention is directed to compounds of Formula (I) wherein

X is Cl.

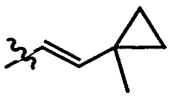
[3] In another embodiment, the present invention is directed to compounds of Formula (I) wherein

R^3 is selected from methyl, ethyl, propyl, i-propyl, and cyclopropyl.

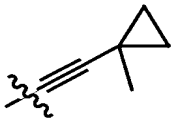
[4] In another embodiment, the present invention is directed to compounds of Formula (I) wherein

R^3 is selected from methyl, ethyl, and cycloproyl.

[5] In another embodiment, the present invention is directed to compounds of Formula (I) wherein

R^2 is  .

[6] In another embodiment, the present invention is directed to compounds of Formula (I) wherein

R^2 is  .

[7] In another embodiment, the present invention is directed to compounds of Formula (I) wherein the compound is selected from:

6-Chloro-3-cyclopropyl-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one;

6-Chloro-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one;

6-Chloro-4-[2-(1-methyl-cyclopropyl)-vinyl]-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one;

6-Chloro-3-methyl-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one; and

6-Chloro-3-ethyl-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one.

The present invention also provides a novel pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount

of a compound of formula (I) or a pharmaceutically acceptable salt form thereof

The compositions and methods of use comprising the compounds of the present invention include stereoisomeric forms thereof, mixtures of stereoisomeric forms thereof, complexes thereof, crystalline forms thereof, prodrug forms thereof and pharmaceutically acceptable salt forms thereof.

In another embodiment, the present invention provides a novel method for treating HIV infection which comprises administering to a host in need of such treatment a therapeutically effective amount of a compound of formula (I) or a pharmaceutically acceptable salt form thereof.

In another embodiment, the present invention provides a novel method of treating HIV infection which comprises administering, in combination, to a host in need thereof a therapeutically effective amount of:

(a) a compound of formula (I); and

(b) at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors.

In another embodiment, the present invention provides a novel method of treating HIV infection which comprises administering, in combination, to a host in need thereof a therapeutically effective amount of:

(a) a compound of formula (I); and

(b) at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors, HIV protease inhibitors, CCR-5 inhibitors, and fusion inhibitors.

Reverse transcriptase inhibitors useful in the above method of treating HIV infection are selected from the group AZT, ddC, ddI, d4T, 3TC, delavirdine, efavirenz, nevirapine, trovirdine, MKC-442, HBY 097, HBY1293, GW867, ACT, UC-781, UC-782, RD4-2025, MEN 10979, and AG1549 (S1153). Protease inhibitors useful in the above method of treating HIV infection are selected from the group saquinavir, ritonavir, indinavir, amprenavir, nelfinavir, palinavir, BMS-232623, GS3333, KNI-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, and ABT-378.

In another embodiment reverse transcriptase inhibitors useful in the above method of treating HIV infection are selected from the group AZT, ddC, ddI, d4T, 3TC, delavirdine, efavirenz, nevirapine, Ro 18,893, trovirdine, MKC-442, HBY 097, HBY1293, GW867, ACT, UC-781, UC-782, RD4-2025, MEN 10979, AG1549 (S1153), TMC-120, TMC-125, Calanolide A, and PMPA. Preferred protease inhibitors useful in the above method of treating HIV infection are selected from the group saquinavir, ritonavir, indinavir, amprenavir, nelfinavir, palinavir, BMS-232623, GS3333, KNI-413, KNI-272, LG-71350, CGP-61755, PD 173606, PD 177298, PD 178390, PD 178392, U-140690, ABT-378, DMP-450, AG-1776, VX-175, MK-944, and VX-478, the CCR-5 inhibitor is selected from TAK-779 (Takeda), SC-351125 (SCH-C, Schering) and SCH-D (Schering), and the fusion inhibitor is selected from T-20 and T1249.

In another embodiment, the reverse transcriptase inhibitor is selected from the group AZT, efavirenz, and 3TC and the protease inhibitor is selected from the group saquinavir, ritonavir, nelfinavir, and indinavir.

In another embodiment, the reverse transcriptase inhibitor is AZT.

In another embodiment, the protease inhibitor is indinavir.

In another embodiment, the present invention provides a pharmaceutical kit useful for the treatment of HIV infection, which comprises a therapeutically effective amount of:

(a) a compound of formula (I); and,

(b) at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors, in one or more sterile containers.

In another embodiment, the present invention provides novel quinazolinones compounds for use in therapy.

In another embodiment, the present invention provides the use of novel quinazolinones compounds for the manufacture of a medicament for the treatment of HIV infection.

The invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof. This invention also encompasses all combinations of preferred aspects of the invention noted herein. It is understood that any and all embodiments of the present invention may be taken in conjunction with any other embodiment to describe additional embodiments of the present invention. Furthermore, any elements of an embodiment are meant to be combined with any and all other elements from any of the embodiments to describe additional embodiments.

DEFINITIONS

It will be appreciated that the compounds of the present invention contain an asymmetrically substituted carbon atom, and may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of racemic forms or by synthesis, from optically active starting materials. All chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure are intended, unless the specific stereochemistry or isomer form is specifically indicated.

The present invention is intended to include all isotopes of atoms occurring on the present compounds. Isotopes include those atoms having the same atomic number but different mass numbers. By way of general example and without limitation, isotopes of hydrogen include tritium and deuterium. Isotopes of carbon include C-13 and C-14.

As used herein, the following terms and expressions have the indicated meanings.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. By way of illustration, the term "C_{1-10} alkyl" or "C_1-C_{10} alkyl" is intended to include C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, and C_{10} alkyl groups. "C_{1-4} alkyl" is intended to include C_1, C_2, C_3, and C_4 alkyl groups. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, n-pentyl, and s-pentyl. "Haloalkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms, substituted with 1 or more halogen (for

example $-C_vF_w$ where v = 1 to 3 and w = 1 to (2v+1)). Examples of haloalkyl include, but are not limited to, trifluoromethyl, trichloromethyl, pentafluoroethyl, and pentachloroethyl. "Alkoxy" represents an alkyl group as defined above with the indicated number of carbon atoms attached through an oxygen bridge. C_{1-10} alkoxy, is intended to include C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, and C_{10} alkoxy groups. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, n-pentoxy, and s-pentoxy. "Cycloalkyl" is intended to include saturated ring groups, such as cyclopropyl, cyclobutyl, or cyclopentyl. C_{3-7} cycloalkyl, is intended to include C_3, C_4, C_5, C_6, and C_7 cycloalkyl groups. "Alkenyl" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl, propenyl and the like. C_{2-10} alkenyl, is intended to include C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, and C_{10} alkenyl groups. "Alkynyl" is intended to include hydrocarbon chains of either a straight or branched configuration and one or more triple carbon-carbon bonds which may occur in any stable point along the chain, such as ethynyl, propynyl and the like. C_{2-10} alkynyl, is intended to include C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, and C_{10} alkynyl groups.

As used herein, "HIV reverse transcriptase inhibitor" is intended to refer to both nucleoside and non-nucleoside inhibitors of HIV reverse transcriptase (RT). Examples of nucleoside RT inhibitors include, but are not limited to, AZT, ddC, ddI, d4T, and 3TC. Examples of non-nucleoside RT inhibitors include, but are no limited to, delavirdine (Pharmacia and Upjohn

U90152S), efavirenz (BMS), nevirapine (Boehringer Ingelheim), Ro 18,893 (Roche), trovirdine (Lilly), MKC-442 (Triangle), HBY 097 (Hoechst), HBY1293 (Hoechst), GW867 (Glaxo Wellcome), ACT (Korean Research Institute), UC-781 (Rega Institute), UC-782 (Rega Institute), RD4-2025 (Tosoh Co. Ltd.), MEN 10979 (Menarini Farmaceutici) and AG1549 (S1153; Agouron)), TMC-120, TMC-125, and Calanolide A.

As used herein, "HIV protease inhibitor" is intended to refer to compounds which inhibit HIV protease. Examples include, but are not limited, saquinavir (Roche, Ro31-8959), ritonavir (Abbott, ABT-538), indinavir (Merck, MK-639), amprenavir (Vertex/Glaxo Wellcome), nelfinavir (Agouron, AG-1343), palinavir (Boehringer Ingelheim), BMS-232623 (Bristol-Myers Squibb), GS3333 (Gilead Sciences), KNI-413 (Japan Energy), KNI-272 (Japan Energy), LG-71350 (LG Chemical), CGP-61755 (Ciba-Geigy), PD 173606 (Parke Davis), PD 177298 (Parke Davis), PD 178390 (Parke Davis), PD 178392 (Parke Davis), U-140690 (Pharmacia and Upjohn), tipranavir (Pharmacia and Upjohn, U-140690), DMP-450 (DuPont), AG-1776, VX-175, MK-944, VX-478 and ABT-378. Additional examples include the cyclic protease inhibitors disclosed in WO93/07128, WO 94/19329, WO 94/22840, and PCT Application Number US96/03426.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric,

hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable benefit/risk ratio.

Since prodrugs are known to enhance numerous desirable qualities of pharmaceuticals (e.g., solubility, bioavailability, manufacturing, etc.) the compounds of the present invention may be delivered in prodrug form. Thus, the present invention is intended to cover prodrugs of the presently claimed compounds, methods of delivering the same and compositions containing the same. "Prodrugs" are intended to include any covalently bonded

carriers that release an active parent drug of the present invention *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs the present invention are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound. Prodrugs include compounds of the present invention wherein a hydroxy, amino, or sulfhydryl group is bonded to any group that, when the prodrug of the present invention is administered to a mammalian subject, it cleaves to form a free hydroxyl, free amino, or free sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol and amine functional groups in the compounds of the present invention. Further examples of prodrugs at are C_{1-6} alkylcarbonyl, C_{1-6} alkoxy, C_{1-4} alkoxycarbonyl, C_{6-10} aryloxy, C_{6-10} aryloxycarbonyl, C_{6-10} arylmethylcarbonyl, C_{1-4} alkylcarbonyloxy C_{1-4} alkoxycarbonyl, C_{6-10} arylcarbonyloxy C_{1-4} alkoxycarbonyl, C_{1-6} alkylaminocarbonyl, phenylaminocarbonyl, and phenyl C_{1-4} alkoxycarbonyl.

"Stable compound" and "stable structure" are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. Only stable compounds are contemplated by the present invention.

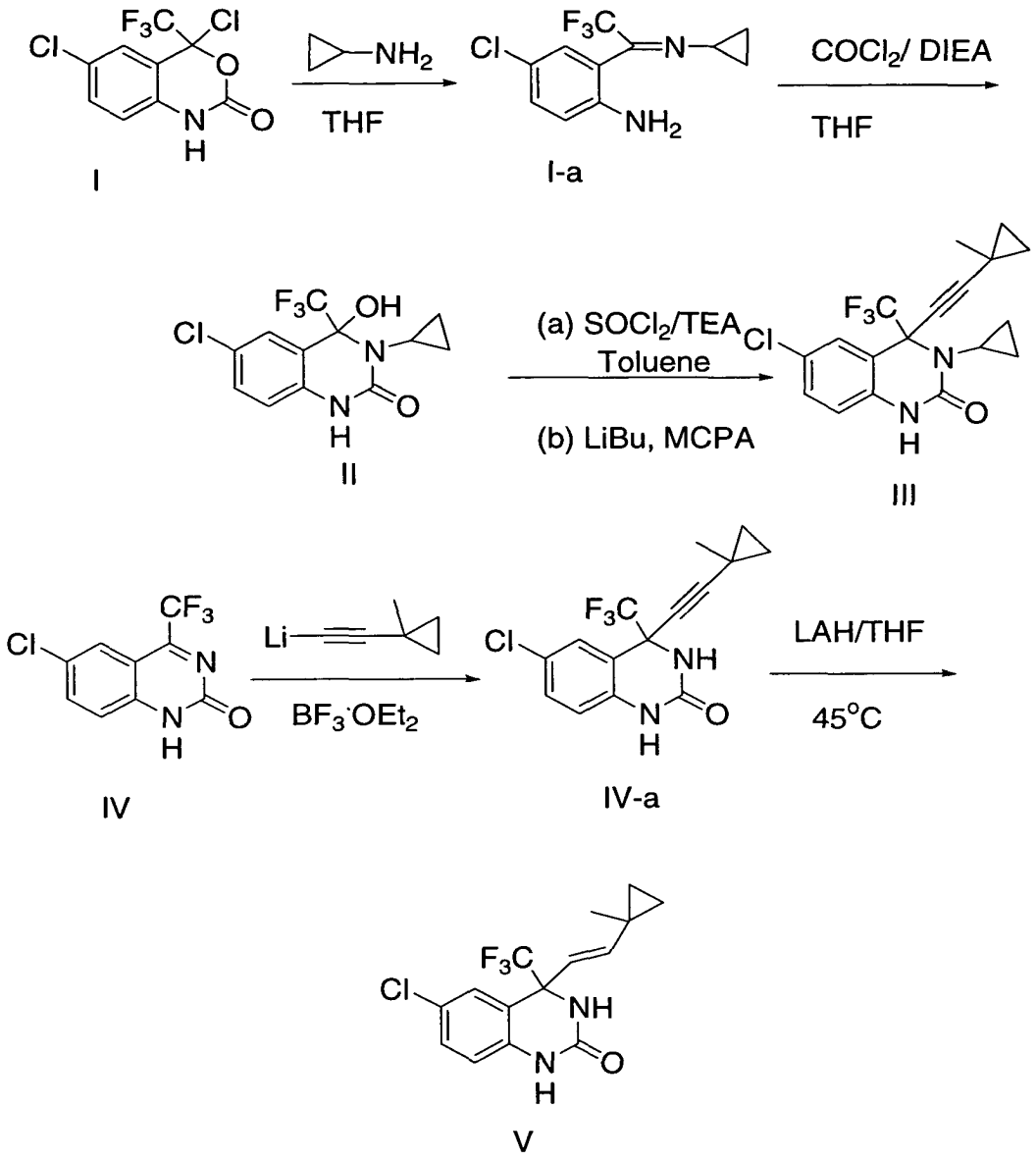
"Therapeutically effective amount" is intended to include an amount of a compound of the present invention alone or in combination with other active ingredients or an amount of the combination of compounds claimed effective to inhibit HIV infection or treat the symptoms of HIV infection in a host. The combination of compounds is preferably a synergistic combination. Synergy, as described for example by Chou and Talalay, Adv. Enzyme Regul. 22:27-55 (1984), occurs when the effect (in this

case, inhibition of HIV replication) of the compounds when administered in combination is greater than the additive effect of the compounds when administered alone as a single agent. In general, a synergistic effect is most clearly demonstrated at suboptimal concentrations of the compounds. Synergy can be in terms of lower cytotoxicity, increased antiviral effect, or some other beneficial effect of the combination compared with the individual components.

As used herein, "treating" or "treatment" cover the treatment of a disease-state in a mammal, particularly in a human, and include: (a) preventing the disease-state from occurring in a mammal, in particular, when such mammal is predisposed to the disease-state but has not yet been diagnosed as having it; (b) inhibiting the disease-state, i.e., arresting its development; and/or (c) relieving the disease-state, i.e., causing regression of the disease state.

Synthesis

The compounds of the present invention can be prepared in a number of ways well known to one skilled in the art of organic synthesis. The compounds of the present invention can be synthesized using the methods described below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. Preferred methods include but are not limited to those methods described below.



EXAMPLE 1

Synthesis of 6-Chloro-3-cyclopropyl-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one (III)

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Part A: A stirring bar equipped , argon flushed 50 mL round bottle was charged with **I** (1.94 g, 6.78 mmol), anhydrous THF (19 mL) and cyclopropylamine (0.97 g, 1.2

mL, 17 mmol), the mixture was stirred at room temperature for 1 hour, then diluted with ethyl acetate, washed with saturated aqueous Na_2CO_3 and saturated aqueous NaCl. The organic phase was dried over $MgSO_4$. Then it was filtered out and concentrated. The resulting oil was dried under reduced pressure to give 1.96 g crude product **I-a**. Y >100%.

Part B: A two neck 100 mL oven dried round bottle was charged with crude **I-a** (1.787 g, 6.78 mmol), anhydrous toluene (34 mL), and DIEA (1.8 mL, 1.3 g, 10 mmol) and the mixture was cooled to 0 °C. To the mixture was added $COCl_2$ (5.4 mL of 1.9 M toluene solution) dropwise. Then ice-bath was removed and the mixture was stirred at room temperature for 60 hours. The reaction was quenched by adding water and diluted with ethyl acetate , the organic phase was separated, washed with saturated aqueous sodium bicarbonate, aqueous sodium chloride and then dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (35-40 % ethyl acetyl acetate in hexane) to give yellow-white solid **II** 1.63 g (78 %).

^{1}HNMR: (acetone-d_6) δ 0.45-0.51 (*m*, 1H, cyclopropyl CH_2), δ0.80-0.89 (*m*, 2H, cyclopropyl CH_2), δ1.08-1.17 (*m*, 1H, cyclopropyl CH_2), δ2.50 (*m*, 1H, cyclopropyl *N-CH*), δ6.80 (*s*, 1H, *OH*), δ6.96-7.00 (*d*, 1H, Aromatic *CH*), δ7.36-7.40 (*dd*, 1H, aromatic *CH*), δ7.49 (*s*, 1H, aromatic *CH*), δ9.08 (*br s*, 1H, *NH*)

^{19}FNMR: (acetone-d_6) 81.78 (*s*, CF_3)

Part C: A 25 mL three neck stirring bar equipped, argon flashed round bottle was charged with **II** (88 mg, 0.29 mmol), anhydrous toluene (1 mL) and the mixture was cooled down to 0 °C. Triethylamine (147 mg, 0.2 mL, 1.45 mmol) was added followed by the dropwise addition of $SOCl_2$ (36 mg, 22 μL, 0.3 mmol). The resulted orange solution was stirred at 0 °C for 30 minutes. And at the same time in another 10mL argon flushed round bottle, methylcyclopropylacetylene (180 μL, 72 mg, 0.9 mmol, 3.1 eq, 40% hexane solution) was dissolved in 0.5 mL THF, and then 1.6 M Butyllithium (0.54 mL, 0.87 mmol, 3 eq) was added. The mixture was stirred in 0 °C ice-bath for 45 min. The resulted lithium methylcyclopropylacetylide solution was cannulated into the above solution of **II** and $SOCl_2$ at 0 °C. After addition, cooling bath was removed and the mixture was stirred at room temperature for 1 hour. The mixture is very cloudy. The reaction was quenched by adding 1M citric acid and dilute with ethyl acetate. The organic phase was separated and washed with saturated aqueous sodium bicarbonate, aqueous sodium chloride and then dried over $MgSO_4$, The solvent was filtered and concentrated. The residue was dissolved in small amount of acetone and the product was crystalized to give 1.02 g **III**. The mother liquid was concentrated and purified by column chromatography (40 % ethyl acetate in hexane) to give 79 mg product **III**. Overall yield 74 %. m.p. 218-220 °C.

^{1}HNMR: (acetone-d_6) δ 0.65-0.70 (*m*, 2H, cyclopropyl CH_2), δ0.78-0.82 (*m*, 2H, cyclopropyl CH_2), δ0.88-1.05 (*m*, 4H, cyclopropyl CH_2), δ1.38 (*s*, 3H, *Me*-cyclopropyl), δ2.55-2.60 (*m*, 1H, cyclopropyl *N-CH*), δ6.96-6.98 (*d*, 1H,

Aromatic *CH*), δ7.37-7.40 (*dd*, 1H, aromatic *CH*), δ7.58 (*s*, 1H, aromatic *CH*), δ9.10 (*br s*, 1H, *NH*)

^{19}FNMR: (acetone-d_6) δ78.37 (*s*, CF_3)

EXAMPLE 2

Synthesis of 6-Chloro-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one (IV-a)

Part A: A 250 mL stir bar equipped, argon flushed three neck round bottle was charged with **IV** (1.50 g, 6.03 mmol), anhydrous THF (30 mL) and the mixture was cooled down to -78 °C. And at the same time in another 100 mL argon flushed round bottle, methylcyclopropylacetylene (4.2 mL, 21.1 mmol, 3.5 eq, 40 % hexane solution) was dissolved in THF (42 mL), and then 1.6 M Butyllithium (12 mL, 19.3 mmol, 3.2 eq) was added. The mixture was stirred in 0 °C ice-bath for 45 min. The resulted lithium methylcyclopropylacetylide solution was cannulated into the above solution of **IV** in THF at -78 °C, then $BF_3 \cdot OEt_2$ (0.43 g, 0.38 mL, 3.0 mmol) was added. After addition, the -78 °C cooling bath was removed and the reaction mixture was stirred at room temperature for 4 hours. The reaction was quenched by adding 1 M citric acid and dilute with ethyl acetate. The organic phase was separated and washed with saturated aqueous sodium bicarbonate, aqueous sodium chloride and then dried over $MgSO_4$, The solvent was filtered and concentrated. The residue was purified by column chromatography (30 % EtOAc in hexane) to give 0.73 g product **IV-a**. (36 %) Another portion 0.26 g was a mixture with product and impurities.

^{1}HNMR: (acetone-d_6) δ 0.72 (*m*, 2H, cyclopropyl CH_2), δ0.95 (m, 2H, cyclopropyl CH_2), δ1.28 (*s*, 3H, *Me*-

cyclopropyl), δ7.00 (*d*, 1H, Aromatic *CH*), δ7.38-7.41 (*dd*, 1H, aromatic *CH*), δ7.50 (*s*, 1H, aromatic *CH*), δ8.98 (*br s*, 1H, *NH*)

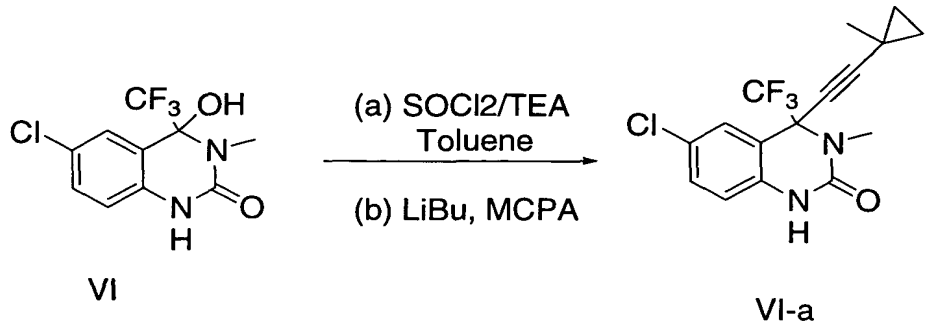
EXAMPLE 3

Synthesis of 6-Chloro-4-[2-(1-methyl-cyclopropyl)-vinyl]-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one (V).

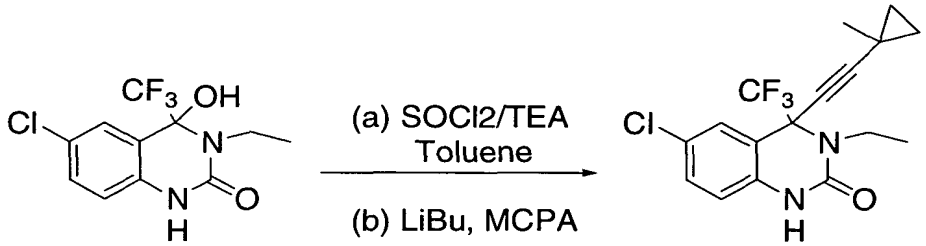
Part A: A 25 ml stir bar and condenser equipped round bottle was charged with **IV-a** (0.73 g, 2.2 mmol), anhydrous THF (4.4 mL), 1,2-dichlorobenzene (0.5 mL, 4.4 mmol, 2 eq) and cooled to 0 °C. Lithium aluminum hydride (6.7 mL, 1.0 M in THF, 3 eq) was slowly added. After addition, the mixture was heated at 45 °C for 16 hours. After the reaction, the mixture was cooled to 0 °C again and quenched by addition of 20 % aqueous $KHSO_4$. The solid was removed by vacuum filtration. The filtrate was diluted with EtOAc and The organic phase was separated and washed with saturated aqueous sodium chloride and then dried over $MgSO_4$, The solvent was filtered and concentrated. The residue was purified by column chromatography (35 % EtOAc in hexane) to give 0.524 g product. (72 %) **V.**

^{1}HNMR: (acetone-d_6) δ 0.64 (*m*, 4H, cyclopropyl CH_2), δ1.16 (*s*, 3H, *Me*-cyclopropyl), δ5.70-5.76 (*d*, 1H, =*CH*), δ6.02-6.07 (*d*, 1H, =*CH*), δ6.89 (*s*, 1H, *NH*), δ7.00 (*d*, 1H, Aromatic *CH*), δ7.30-7.33 (*dd*, 1H, aromatic *CH*), δ7.42 (*s*, 1H, aromatic *CH*), δ8.95 (*br s*, 1H, *NH*)

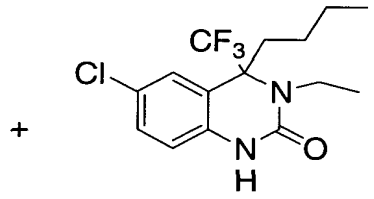
^{19}FNMR: δ81.71 (*s*, CF_3)



VI VI-a



VII VII-a



VIII

EXAMPLE 4

Synthesis of 6-Chloro-3-methyl-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one (VI-a).

A 25 ml three neck stirring bar equipped, argon flushed round bottle was charged with **VI** (85 mg, 0.3 mmol), anhydrous toluene (1 mL) and the mixture was cooled down to 0 °C. Triethylamine (152 mg, 210 µL 1.5 mmol, 5 eq) was added followed by the drop-wise addition

of $SOCl_2$ (40 mg, 24 μL 0.315 mmol, 1.05 eq). The resulted orange solution was stirred at 0 °C for 30 minutes. And at the same time in another 10 mL argon flushed round bottle, methylcyclopropylacetylene (186 μL, 0.93 mmol, 3.1 eq) was dissolved in 0.5 mL THF, and then 1.6 M Butyllithium (0.58 mL, solution in hexane) was added. The mixture was stirred in 0 °C ice-bath for 45 min. The resulted lithium methylcyclopropylacetylide solution was cannulated into the above solution of **VI** and $SOCl_2$ at 0 °C. After addition, cooling bath was removed and the mixture was stirred at room temperature for 2.5 hour. The reaction was quenched by adding 1 M citric acid and diluted with ethyl acetate. The organic phase was separated and washed with saturated aqueous sodium bicarbonate, aqueous sodium chloride and then dried over $MgSO_4$. The solvent was filtered and concentrated. The residue was purified by column chromatography (30 % ethyl acetate in hexane) to give 44 mg product **VI-a**. yield 43 %, m.p.206.1-208.3 °C.

^{1}HNMR: (acetone-d_6) δ 0.78-0.80 (*m*, 2H, cyclopropyl CH_2), δ1.03 (m, 2H, cyclopropyl CH_2), δ1.35(*s*, 3H, *Me*-cyclopropyl), δ3.2 (*s*, 3H, $\underline{CH_3}$), δ7.0 (*d*, 1H, Aromatic *CH*), δ7.38-7.42 (*dd*, 1H, aromatic *CH*), δ7.54 (*s*, 1H, aromatic *CH*), δ9.08 (*br s*, 1H, *NH*)

^{19}FNMR: (acetone-d_6) δ79.25 (*s*, CF_3)

EXAMPLE 5

Synthesis of 6-Chloro-3-ethyl-4-(1-methyl-cyclopropylethynyl)-4-trifluoromethyl-3,4-dihydro-1H-quinazolin-2-one (VII-a).

A 25 ml three neck stirring bar equipped, argon flushed round bottle was charged with **VII** (89 mg, 0.3 mmol), anhydrous toluene (1 mL) and the mixture was cooled down to 0 °C. Triethylamine (152 mg, 210 µL, 1.5 mmol, 5 eq) was added followed by the dropwise addition of $SOCl_2$ (40 mg, 24 µL, 0.315 mmol, 1.05 eq). The resulted orange solution was stirred at 0 °C for 30 minutes. And at the same time in another 10 mL argon flushed round bottle, methylcyclopropylacetylene (186 µL, 0.93 mmol, 3.1 eq) was dissolved in 0.5 mL THF, and then 1.6 M Butyllithium (0.58 mL, solution in hexane) was added. The mixture was stirred in 0 °C ice-bath for 45 min. The resulted lithium methylcyclopropylacetylide solution was cannulated into the above THF solution of **VII** and $SOCl_2$ at 0 °C. After addition, cooling bath was removed and the mixture was stirred at room temperature for 2.5 hour. The reaction was quenched by adding 1 M citric acid and diluted with ethyl acetate. The organic phase was separated and washed with saturated aqueous sodium bicarbonate, aqueous sodium chloride and then dried over $MgSO_4$, The solvent was filtered and concentrated. The residue was purified by column chromatography (30 % ethyl acetate in hexane) to give 17 mg product **VII-a**. yield 16 %. An undesired side product **VIII** was in about 30 %yield, m.p. 218.7-221.8 °C

^{1}HNMR: (acetone-d_6) δ 0.78-0.80 (*m*, 2H, cyclopropyl CH_2), δ1.03 (*m*, 2H, cyclopropyl CH_2), δ1.20 (*t*, 3H, $CH_2\underline{CH_3}$), δ1.36 (*s*, 3H, *Me*-cyclopropyl), δ3.5-3.6 (*m*, 1H, $\underline{CH_2}CH_3$), δ3.9-4.0 (*m*, 1H, $\underline{CH_2}CH_3$), δ7.0 (*d*, 1H, Aromatic *CH*), δ7.38-7.42 (*dd*, 1H, aromatic *CH*), δ7.53 (*s*, 1H, aromatic *CH*), δ9.04 (*bs*, 1H, *NH*)

^{19}FNMR: (acetone-d_6) 80.5 (*s*, CF_3)

Utility

The compounds of this invention possess reverse transcriptase inhibitory activity and HIV inhibitory efficacy. The compounds of formula (I) possess HIV reverse transcriptase inhibitory activity and are therefore useful as antiviral agents for the treatment of HIV infection and associated diseases. The compounds of formula (I) possess HIV reverse transcriptase inhibitory activity and are effective as inhibitors of HIV growth. The ability of the compounds of the present invention to inhibit viral growth or infectivity is demonstrated in standard assay of viral growth or infectivity, for example, using the assay described below.

The compounds of formula (I) of the present invention are also useful for the inhibition of HIV in an *ex vivo* sample containing HIV or expected to be exposed to HIV. Thus, the compounds of the present invention may be used to inhibit HIV present in a body fluid sample (for example, a serum or semen sample) which contains or is suspected to contain or be exposed to HIV.

The compounds provided by this invention are also useful as standard or reference compounds for use in tests or assays for determining the ability of an agent to inhibit viral replication and/or HIV reverse transcriptase, for example in a pharmaceutical research program. Thus, the compounds of the present invention may be used as a control or reference compound in such assays and as a quality control standard. The compounds of the present invention may be provided in a commercial kit or container for use as such standard or reference compound.

Since the compounds of the present invention exhibit specificity for HIV reverse transcriptase, the compounds of the present invention may also be useful as diagnostic reagents in diagnostic assays for the detection of HIV reverse transcriptase. Thus, inhibition of the reverse transcriptase activity in an assay (such as the assays described herein) by a compound of the present invention would be indicative of the presence of HIV reverse transcriptase and HIV virus.

As used herein "μg" denotes microgram, "mg" denotes milligram, "g" denotes gram, "μL" denotes microliter, "mL" denotes milliliter, "L" denotes liter, "nM" denotes nanomolar, "μM" denotes micromolar, "mM" denotes millimolar, "M" denotes molar and "nm" denotes nanometer. "Sigma" stands for the Sigma-Aldrich Corp. of St. Louis, MO.

Compounds tested in the assay described below are considered to be active if they exhibit a K_i of $\leq$10 μM. Compounds of the present invention have K_i's of $\leq$0.01 μM or have K_i's of $\leq$0.001 μM.

Using the methodology described below, a number of compounds of the present invention were found to exhibit a K_i of $\leq$10 μM, thereby confirming the utility of the compounds of the present invention as effective HIV reverse transcriptase inhibitors.

HIV RNA Assay

DNA Plasmids and *in vitro* RNA transcripts:

Plasmid pDAB 72 containing both gag and pol sequences of BH10 (bp 113-1816) cloned into PTZ 19R was prepared according to Erickson-Viitanen et al. *AIDS Research and Human Retroviruses* **1989**, *5*, 577. The plasmid was linearized with Bam HI prior to the generation of *in vitro* RNA transcripts using the

Riboprobe Gemini system II kit (Promega) with T7 RNA polymerase. Synthesized RNA was purified by treatment with RNase free DNAse (Promega), phenol-chloroform extraction, and ethanol precipitation. RNA transcripts were dissolved in water, and stored at -70°C. The concentration of RNA was determined from the A_{260}.

Probes:

Biotinylated capture probes were purified by HPLC after synthesis on an Applied Biosystems (Foster City, CA) DNA synthesizer by addition of biotin to the 5' terminal end of the oligonucleotide, using the biotin-phosphoramidite reagent of Cocuzza, *Tet. Lett.* **1989**, *30*, 6287. The gag biotinylated capture probe (as described in WO01/29037, published April 26, 2001) was complementary to nucleotides 889-912 of HXB2 and the pol biotinylated capture probe (see WO01/29037) was complementary to nucleotides 2374-2395 of HXB2. Alkaline phosphatase conjugated oligonucleotides used as reporter probes were prepared by Syngene (San Diego, CA.). The pol reporter probe (see WO01/29037) was complementary to nucleotides 2403-2425 of HXB2. The gag reporter probe (see WO01/29037) was complementary to nucleotides 950-973 of HXB2. All nucleotide positions are those of the GenBank Genetic Sequence Data Bank as accessed through the Genetics Computer Group Sequence Analysis Software Package (Devereau *Nucleic Acids Research* **1984**, *12*, 387). The reporter probes were prepared as 0.5 µM stocks in 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate), 0.05 M Tris pH 8.8, 1 mg/mL BSA. The biotinylated capture probes were prepared as 100 µM stocks in water.

Streptavidin coated plates:

Streptavidin coated plates were obtained from DuPont Biotechnology Systems (Boston, MA).

Cells and virus stocks:

MT-2 and MT-4 cells were maintained in RPMI 1640 supplemented with 5% fetal calf serum (FCS) for MT-2 cells or 10% FCS for MT-4 cells, 2 mM L-glutamine and 50 µg/mL gentamycin, all from Gibco. HIV-1 RF was propagated in MT-4 cells in the same medium. Virus stocks were prepared approximately 10 days after acute infection of MT-4 cells and stored as aliquots at -70°C. Infectious titers of HIV-1(RF) stocks were 1-3 x 10^7 PFU (plaque forming units)/mL as measured by plaque assay on MT-2 cells (see below). Each aliquot of virus stock used for infection was thawed only once.

For evaluation of antiviral efficacy, cells to be infected were subcultured one day prior to infection. On the day of infection, cells were resuspended at 5 x 10^5 cells/mL in RPMI 1640, 5% FCS for bulk infections or at 2 x 10^6/mL in Dulbecco's modified Eagles medium with 5% FCS for infection in microtiter plates. Virus was added and culture continued for 3 days at 37°C.

HIV RNA assay:

Cell lysates or purified RNA in 3 M or 5 M GED were mixed with 5 M GED and capture probe to a final guanidinium isothiocyanate concentration of 3 M and a final biotin oligonucleotide concentration of 30 nM. Hybridization was carried out in sealed U bottom 96 well tissue culture plates (Nunc or Costar) for 16-20 hours at 37°C. RNA hybridization reactions were diluted three-fold with deionized water to a final guanidinium

isothiocyanate concentration of 1 M and aliquots (150 µL) were transferred to streptavidin coated microtiter plates wells. Binding of capture probe and capture probe-RNA hybrid to the immobilized streptavidin was allowed to proceed for 2 hours at room temperature, after which the plates were washed 6 times with DuPont ELISA plate wash buffer (phosphate buffered saline(PBS), 0.05% Tween 20) A second hybridization of reporter probe to the immobilized complex of capture probe and hybridized target RNA was carried out in the washed streptavidin coated well by addition of 120 µl of a hybridization cocktail containing 4 X SSC, 0.66% Triton X 100, 6.66% deionized formamide, 1 mg/mL BSA and 5 nM reporter probe. After hybridization for one hour at 37°C, the plate was again washed 6 times. Immobilized alkaline phosphatase activity was detected by addition of 100 µL of 0.2 mM 4-methylumbelliferyl phosphate (MUBP, JBL Scientific) in buffer (2.5 M diethanolamine pH 8.9 (JBL Scientific), 10 mM $MgCl_2$, 5 mM zinc acetate dihydrate and 5 mM *N*-hydroxyethyl-ethylene-diamine-triacetic acid). The plates were incubated at 37°C. Fluorescence at 450 nM was measured using a microplate fluorometer (Dynateck) exciting at 365 nM.

Microplate based compound evaluation in HIV-1 infected MT-2 cells:

Compounds to be evaluated were dissolved in DMSO and diluted in culture medium to twice the highest concentration to be tested and a maximum DMSO concentration of 2%. Further three-fold serial dilutions of the compound in culture medium were performed directly in U bottom microtiter plates (Nunc). After compound dilution, MT-2 cells (50 µL) were added to a final

concentration of 5 x 10^5 per mL (1 x 10^5 per well). Cells were incubated with compounds for 30 minutes at 37°C in a CO_2 incubator. For evaluation of antiviral potency, an appropriate dilution of HIV-1 (RF) virus stock (50 µL) was added to culture wells containing cells and dilutions of the test compounds. The final volume in each well was 200 µL. Eight wells per plate were left uninfected with 50 µL of medium added in place of virus, while eight wells were infected in the absence of any antiviral compound. For evaluation of compound toxicity, parallel plates were cultured without virus infection.

After 3 days of culture at 37°C in a humidified chamber inside a CO_2 incubator, all but 25 µL of medium/well was removed from the HIV infected plates. Thirty seven µL of 5 M GED containing biotinylated capture probe was added to the settled cells and remaining medium in each well to a final concentration of 3 M GED and 30 nM capture probe. Hybridization of the capture probe to HIV RNA in the cell lysate was carried out in the same microplate well used for virus culture by sealing the plate with a plate sealer (Costar), and incubating for 16-20 hrs in a 37°C incubator. Distilled water was then added to each well to dilute the hybridization reaction three-fold and 150 µL of this diluted mixture was transferred to a streptavidin coated microtiter plate. HIV RNA was quantitated as described above. A standard curve, prepared by adding known amounts of pDAB 72 *in vitro* RNA transcript to wells containing lysed uninfected cells, was run on each microtiter plate in order to determine the amount of viral RNA made during the infection.

In order to standardize the virus inoculum used in the evaluation of compounds for antiviral activity,

dilutions of virus were selected which resulted in an IC_{90} value (concentration of compound required to reduce the HIV RNA level by 90%) for dideoxycytidine (ddC) of 0.2 µg/mL. IC_{90} values of other antiviral compounds, both more and less potent than ddC, were reproducible using several stocks of HIV-1 (RF) when this procedure was followed. This concentration of virus corresponded to ~3 x 10^5 PFU (measured by plaque assay on MT-2 cells) per assay well and typically produced approximately 75% of the maximum viral RNA level achievable at any virus inoculum. For the HIV RNA assay, IC_{90} values were determined from the percent reduction of net signal (signal from infected cell samples minus signal from uninfected cell samples) in the RNA assay relative to the net signal from infected, untreated cells on the same culture plate (average of eight wells). Valid performance of individual infection and RNA assay tests was judged according to three criteria. It was required that the virus infection should result in an RNA assay signal equal to or greater than the signal generated from 2 ng of pDAB 72 *in vitro* RNA transcript. The IC_{90} for ddC, determined in each assay run, should be between 0.1 and 0.3 µg/mL. Finally, the plateau level of viral RNA produced by an effective reverse transcriptase inhibitor should be less than 10% of the level achieved in an uninhibited infection. A compound was considered active if its IC_{90} was found to be less than 20µM.

For antiviral potency tests, all manipulations in microtiter plates, following the initial addition of 2X concentrated compound solution to a single row of wells, were performed using a Perkin Elmer/Cetus ProPette.

Protein Binding and Mutant Resistance

In order to characterize NNRTI compounds for their clinical efficacy potential the effect of plasma proteins on antiviral potency and measurements of antiviral potency against wild type and mutant variants of HIV which carry amino acid changes in the known binding site for NNRTIs were examined. The rationale for this testing strategy is two fold:

1. Many drugs are extensively bound to plasma proteins. Although the binding affinity for most drugs for the major components of human plasma, namely, human serum albumin (HSA) or alpha-1-acid glycoprotein (AAG), is low, these major components are present in high concentration in the blood. Only free or unbound drug is available to cross the infected cell membrane for interaction with the target site (i.e., HIV-1 reverse transcriptase, HIV-1 RT). Therefore, the effect of added HSA+AAG on the antiviral potency in tissue culture more closely reflects the potency of a given compound in the clinical setting. The concentration of compound required for 90% inhibition of virus replication as measured in a sensitive viral RNA-based detection method is designated the IC90. The fold increase in apparent IC90 for test compounds in the presence or added levels of HSA and AAG that reflect *in vivo* concentrations (45 mg/ml HSA, 1 mg/ml AAG) was then calculated. The lower the fold increase, the more compound will be available to interact with the target site.

2. The combination of the high rate of virus replication in the infected individual and the poor fidelity of the viral RT results in the production of a quasi-species or mixtures of HIV species in the infected individual. These species will include a majority wild

type species, but also mutant variants of HIV and the proportion of a given mutant will reflect its relative fitness and replication rate. Because mutant variants including mutants with changes in the amino acid sequence of the viral RT likely pre-exist in the infected individual's quasi-species, the overall potency observed in the clinical setting will reflect the ability of a drug to inhibit not only wild type HIV-1, but mutant variants as well. We thus have constructed, in a known genetic background, mutant variants of HIV-1 which carry amino acid substitutions at positions thought to be involved in NNRTI binding, and measured the ability of test compounds to inhibit replication of these mutant viruses. The concentration of compound required for 90% inhibition of virus replication as measured in a sensitive viral RNA-based detection method is designated the IC90. It is desirable to have a compound which has high activity against a variety of mutants.

The protein binding for the compound was also measured in an assay designed to solely measure protein binding. The *in vitro* protein binding of the compound of the present invention was determined by equilibrium dialysis using pooled human serum. The compound as added to human serum to achieve a final concentration of 10 μM. An aliquot of the spiked serum was then loaded to one side of an assembled equilibrium dialysis teflon cell, while potassium phosphate buffer (0.133M, pH 7.4) was loaded to the other side of the cell. After incubation at 37°C , the serum and buffer samples were collected, extracted and the compound concentrations determined by liquid chromatography mass spectral analysis. The percent of drug unbound was calculated by dividing the

buffer concentration by the serum concentration and then multiplying by 100.

The compounds of the present invention have sufficient protein binding free fraction and pharmacokinetics in the chimp to exceed the IC90s of the clinically relevant mutant HIV-1 viruses in vivo. The plasma IC90 may be calculated as described in Corbett, et al., J. Med. Chem. (2000), 43, 2019-2030, which is herein incorporated by reference.

The compounds of the present invention may also have improved pharmacokinectic properties.

Dosage and Formulation

The antiviral compounds of this invention can be administered as treatment for viral infections by any means that produces contact of the active agent with the agent's site of action, i.e., the viral reverse transcriptase, in the body of a mammal. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. They can be administered alone, but preferably are administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the age, health and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient can be expected to be about 0.001 to about 1000 milligrams per kilogram of body weight, with the preferred dose being about 0.1 to about 30 mg/kg.

Dosage forms of compositions suitable for administration contain from about 1 mg to about 100 mg of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition. The active ingredient can be administered orally in solid dosage forms, such as capsules, tablets and powders, or in liquid dosage forms, such as elixirs, syrups and suspensions. It can also be administered parenterally, in sterile liquid dosage forms.

Gelatin capsules contain the active ingredient and powdered carriers, such as lactose, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts, and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben

and chlorobutanol. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences, supra,* a standard reference text in this field.

Useful pharmaceutical dosage-forms for administration of the compounds of this invention can be illustrated as follows:

Capsules

A capsule formulation of the present invention can be prepared by filling standard two-piece hard gelatin capsules each with 100 mg of powdered active ingredient, 150 mg of lactose, 50 mg of cellulose, and 6 mg magnesium stearic.

Soft Gelatin Capsules

A soft gelatin capsule formulation of the present invention can be prepared as follows. A mixture of active ingredient in a digestible oil such as soybean oil, cottonseed oil or olive oil can be prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 100 mg of the active ingredient. The capsules should then be washed and dried.

Tablets

A tablet formulation of the present invention can be prepared by conventional procedures so that the dosage unit is 100 mg of active ingredient, 0.2 mg of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 mg of microcrystalline cellulose, 11 mg of starch and 98.8 mg of lactose. Appropriate coatings may be applied to increase palatability or delay absorption.

Suspension

An aqueous suspension formulation can be prepared for oral administration so that each 5 mL contain 25 mg of finely divided active ingredient, 200 mg of sodium

carboxymethyl cellulose, 5 mg of sodium benzoate, 1.0 g of sorbitol solution, U.S.P., and 0.025 mg of vanillin.

Injectable

A parenteral formulation suitable for administration by injection can be prepared by stirring 1.5% by weight of active ingredient in 10% by volume propylene glycol and water. The solution is sterilized by commonly used techniques.

Combination Administration of Therapeutic Agents

The present invention provides a method for the treatment of HIV infection which comprises administering, in combination, to a host in need thereof a therapeutically effective amount of the following:

(a) a compound of formula (I); and

(b) at least one compound selected from the group consisting of HIV reverse transcriptase inhibitors and HIV protease inhibitors, in one or more sterile containers.

Each therapeutic agent component of this combination method (i.e., component (a) and (b) set forth above) can independently be administered in any separate dosage form, such as those described above, and can be administered in various ways, as described above. In the following description component (b) is to be understood to represent one or more agents as described previously. Each individual therapeutic agent comprising component (b) may also be independently be administered in any separate dosage form, such as those described above, and can be administered in various ways, as described above.

Components (a) and any one or more of the agents comprising component (b) of the combination method of the present invention may be formulated together, in a single

dosage unit (that is, combined together in one capsule, tablet, powder, or liquid, etc.) as a combination product. When component (a) and (b) are not formulated together in a single dosage unit, the component (a) may be administered at the same time as component (b) or in any order; for example component (a) of this invention may be administered first, followed by administration of component (b), or they may be administered in the revserse order. If component (b) contains more that one agent, e.g., one RT inhibitor and one protease inhibitor, these agents may be administered together or in any order. When not administered at the same time, preferably the administration of component (a) and (b) occurs less than about one hour apart. Preferably, the route of administration of component (a) and (b) is oral. The terms oral agent, oral inhibitor, oral compound, or the like, as used herein, denote compounds which may be orally administered. Although it is preferable that component (a) and component (b) both be administered by the same route (that is, for example, both orally) or dosage form, if desired, they may each be administered by different routes or dosage forms (for example, one component of the combination method may be administered orally, and another component may be administered intravenously).

As is appreciated by a medical practitioner skilled in the art, the dosage of the combination therapy of the invention may vary depending upon various factors such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the kind of concurrent treatment, the

frequency of treatment, and the effect desired, as described above.

The proper dosage of components (a) and (b) of the combination method of this invention will be readily ascertainable by a medical practitioner skilled in the art, based upon the present disclosure. By way of general guidance, typically a daily dosage may be about 100 milligrams to about 1.5 grams of each component. If component (b) represents more than one compound, then typically a daily dosage may be about 100 milligrams to about 1.5 grams of each agent of component (b). By way of general guidance, when the compounds of component (a) and component (b) are administered in combination, the dosage amount of each component may be reduced by about 70-80% relative to the usual dosage of the component when it is administered alone as a single agent for the treatment of HIV infection, in view of the synergistic effect of the combination.

The combination products of this invention may be formulated such that, although the active ingredients are combined in a single dosage unit, the physical contact between the active ingredients is minimized. In order to minimize contact, for example, where the product is orally administered, one active ingredient may be enteric coated. By enteric coating one of the active ingredients, it is possible not only to minimize the contact between the combined active ingredients, but also, it is possible to control the release of one of these components in the gastrointestinal tract such that one of these components is not released in the stomach but rather is released in the intestines. Another embodiment of this invention where oral administration is desired provides for a combination product wherein one of

the active ingredients is coated with a sustained-release material which effects a sustained-release throughout the gastrointestinal tract and also serves to minimize physical contact between the combined active ingredients. Furthermore, the sustained-released component can be additionally enteric coated such that the release of this component occurs only in the intestine. Still another approach would involve the formulation of a combination product in which the one component is coated with a sustained and/or enteric release polymer, and the other component is also coated with a polymer such as a low-viscosity grade of hydroxypropyl methylcellulose or other appropriate materials as known in the art, in order to further separate the active components. The polymer coating serves to form an additional barrier to interaction with the other component. In each formulation wherein contact is prevented between components (a) and (b) via a coating or some other material, contact may also be prevented between the individual agents of component (b).

Dosage forms of the combination products of the present invention wherein one active ingredient is enteric coated can be in the form of tablets such that the enteric coated component and the other active ingredient are blended together and then compressed into a tablet or such that the enteric coated component is compressed into one tablet layer and the other active ingredient is compressed into an additional layer. Optionally, in order to further separate the two layers, one or more placebo layers may be present such that the placebo layer is between the layers of active ingredients. In addition, dosage forms of the present invention can be in the form of capsules wherein one

active ingredient is compressed into a tablet or in the form of a plurality of microtablets, particles, granules or non-perils, which are then enteric coated. These enteric coated microtablets, particles, granules or non-perils are then placed into a capsule or compressed into a capsule along with a granulation of the other active ingredient.

These as well as other ways of minimizing contact between the components of combination products of the present invention, whether administered in a single dosage form or administered in separate forms but at the same time or concurrently by the same manner, will be readily apparent to those skilled in the art, based on the present disclosure.

Pharmaceutical kits useful for the treatment of HIV infection, which comprise a therapeutically effective amount of a pharmaceutical composition comprising a compound of component (a) and one or more compounds of component (b), in one or more sterile containers, are also within the ambit of the present invention. Sterilization of the container may be carried out using conventional sterilization methodology well known to those skilled in the art. Component (a) and component (b) may be in the same sterile container or in separate sterile containers. The sterile containers of materials may comprise separate containers, or one or more multi-part containers, as desired. Component (a) and component (b) may be separate, or physically combined into a single dosage form or unit as described above. Such kits may further include, if desired, one or more of various conventional pharmaceutical kit components, such as for example, one or more pharmaceutically acceptable carriers, additional vials for mixing the components,

etc., as will be readily apparent to those skilled in the art. Instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, may also be included in the kit.

As will be appreciated by one of skill in the art, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.